Crystallization and structure analysis of a PsbM-deletion mutant of PSII  
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Photosystem II (PSII) is a membrane protein complex located in the thylakoid membranes of oxygenic photosynthetic organisms, and performs light-induced oxygen evolution from water. PSII consists of four large trans-membrane (TM) subunits, thirteen small TM subunits and three peripheral hydrophilic subunits. PsbM is one of the small TM subunits of PSII, and is located in the monomer-monomer interface of PSII dimer. The functions of PsbM include stabilization of the PSII dimer and the plastoquinone (Q) binding site. In order to elucidate the structural basis for PsbM function, we purified and crystallized oxygen-evolving PSII core complex from a PsbM-deletion mutant of Thermosynechococcus vulcanus, and started its crystallographic analysis.

Mutant cells were grown in a phosphoric acid culture media. The mutant PSII dimer was purified by anion exchange chromatography after LDAO and β-DDM solubilization with slight modifications described in [1]. Crystallization was carried out with an oil-batch method, and the crystals obtained were soaked into a cryoprotectant solution followed by an exposure to air for dehydration before flash-cooling in a nitrogen gas stream at 100K. X-ray diffraction images were collected at BL41XU of SPring-8. The diffraction data was processed to 2.45 Å resolution by mosflm, and structure analysis was carried out with the molecular replacement method with the wild type PSII structure refined at 1.9 Å resolution as a search model [2]. From the results obtained, we confirmed the lack of PsbM in the Fm – Fv/v0 isomorph difference Fourier map calculated with the phase information of the wild-type crystal, and found small but significant differences between the subunit structures of mutant and wild type PSII based on the structural model obtained, and structural changes may be expected to be visible after the structure refinement is completed at 2.45 Å resolution.  


Keywords: photosynthesis, crystal structure, mutant

Crystal structure of the STAS domain of the SLC26 anion transporter prestin  
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The SuP superfamily of anion transporters includes over 200 sequenced members from archaea to mammals. Among these, the members of the mammalian SLC26 family are multipurpose anion exchangers with fundamental roles in human physiology; many of them are involved in genetic diseases. As an extreme example of versatility, prestin (SLC26A5) works as a voltage-driven membrane motor rather than a transporter, and it is responsible for the outer hair cells somatic electromotility that increases hearing sensitivity and frequency selectivity in mammals [1]. In stark contrast with the wealth of information accumulated on cationic transport proteins, the structure and transport mechanisms of anion transporters remain largely unexplored, the only exception being CIC chloride channels. SuP/SLC26 anion transporters carry a hydrophobic core with a variable membrane topology and a C-terminal cytosolic domain (around 240 amino acids long in prestin) that is essential in plasma membrane targeting and protein function. In prestin, deletions, mutations or replacement with analogous C-terminal portions of other SLC26 family members of the mammalian SLC26 family are multipurpose anion transporters, the

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A C-Terminal Pathway in the calcium pump  
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The sarco(endo)plasmic reticulum calcium pump (SERCA) is an integral membrane protein of the P-type ATPase family, responsible for calcium transport from the cytoplasm into the sarco- or endoplasmic reticulum. Its activity, maintaining low (micromolar) calcium levels in the cytoplasm while re-filling intracellular calcium stores, is vital for muscle function and calcium signaling processes.

We have solved a 2.2 Å crystal structure of SERCA 1a from rabbit hind leg muscle, stabilized by thapsigargin and aluminium fluoride in the calcium-free transition state of dephosphorylation. The relatively high resolution and low mosaicity data of this crystal form allow us to locate several well-defined water molecules within the membrane-embedded region of the enzyme, pinpointing a hydrated cavity close to the C-terminus of the enzyme. A systematic comparison of SERCA crystal structures in six different catalytic states reveals that this cavity is exclusive to calcium-free states. Molecular dynamics simulations confirm that the C-terminal cavity constitutes an open, fully hydrated connecting path between the ion binding sites and the cytoplasm in calcium-free SERCA. In agreement with mutational studies [1-4], and in analogy to a similar pathway recently described in the related sodium-potassium pump [5], we suggest that this C-terminal pathway plays a functional role in proton and/or calcium exchange with the cytoplasm.

Keywords: membrane, transport, ATPase

Poster Sessions